NHEERL Publications Available Online
March 19-25, 2018

ORD-024226 Adv Notification	Conley, J., C. Lambright, N. Evans, M. Cardon, J. Furr, V. Wilson, and E. Gray. Mixed "anti-androgenic" chemicals at low individual doses produces reproductive tract malformations in the male rat. TOXICOLOGICAL SCIENCES. [HYPERLINK "https://doi.org/10.1093/toxsci/kfy069"]	Earl Gray, ord, nheerl, tad	Peer Reviewed	CSS 17.01.01

Cleared in STICS 1/3/2018. Now available online.

Impact / Purpose Statement

The USEPA is required by federal law (Food Quality Protection Act, Safe Drinking Water Act) to assess the cumulative risk of exposure to chemicals that are toxicologically similar. Thus far Agency efforts have focused on grouping chemicals for cumulative risk assessment based on chemical similarity (e.g., organophosphate pesticides). Research from our group, detailed in the present manuscript, demonstrates the need to more broadly group chemicals that target a similar biological signaling pathway - in this case, fetal male reproductive development coordinated via the androgen receptor. Further, we demonstrate the use of Adverse Outcome Pathway networks (AOPn) for identifying chemicals with disparate MIEs that target a common pathway and may be suitable for cumulative risk assessment.

Product Description / Abstract

Journal article abstract follows: Biomonitoring efforts have clearly shown that all humans are exposed to chemical mixtures. Of concern is whether or not exposure to mixtures during pregnancy contributes to congenital abnormalities in children even when each chemical is at an individual dose that does not affect the fetus. Here, we hypothesized that in utero exposure to a mixture of chemicals covering multiple “anti-androgenic” mechanisms of action at doses that have no adverse effect in utero would result in permanent reproductive tract alterations in the male rat after birth. Pregnant dams were exposed to a range of dilutions (100, 50, 25, 12.5, 6.25%, or control) of a mixture containing pesticides, phthalates and drugs (p,p’-DDE, linuron, prochloraz, procymidone, pyrifluquinazon, vinclozolin, finasteride, flutamide, simvastatin, and 9 phthalates (dipentyl, dicyclohexyl, di-2-ethylhexyl, dibutyl, benzyl butyl, diisobutyl, diisoheptyl, dihexyl, and diheptyl)). The top dose contained each chemical at 20% of its lowest observed adverse effect level (LOAEL) for the most sensitive male reproductive alteration following in utero exposure. We found that male rat offspring displayed a variety of neonatal, pubertal, and permanent adult effects across all dose levels. Even at the lowest dose (each chemical ~80-fold below LOAEL) there were permanent reductions in several reproductive tract tissue weights. In the top dose group, 100% of male offspring displayed permanent severe birth defects including genital malformations. Despite multiple mechanisms of action, exposure during pregnancy to a mixture of 18 chemicals at doses 80-fold below the individual chemical LOAELS resulted in permanent, congenital reproductive alterations

ORD-023037	Cheng, W., K. Duncan, A. Ghio, C. Ward-Caviness, E. Karoly, D. Diaz-Sanchez, R. Conolly, and R. Devlin. Changes in Metabolites Present in Lung Lining Fluid Following Exposure of Humans to Ozone. TOXICOLOGICAL SCIENCES. [HYPERLINK "https://doi.org/10.1093/toxsci/kfy043"]	Robert Devlin, ord, nheerl, ephd	Peer Reviewed	ACE PEP-1.1

Cleared in STICS 8/18/2017. Now available online.

Impact / Purpose Statement

This study was done to better understand the biological pathways impacted by exposure of humans to ozone. Low molecular weight metabolites present in blood were assessed following controlled exposure to clean and ozone. Significant changes were observed in pathways involving anti-oxidant response, altered energy metabolism, lipid membrane turnover, and amino acid metabolism. This is the first study in humans to characterize metabolic pathways affected by ozone.

Product Description / Abstract

Controlled human exposure to the oxidant air pollutant ozone causes decrements in lung function and increased inflammation as evidenced by neutrophil influx into the lung and increased levels of proinflammatory cytokines in the airways. Here we describe a targeted metabolomics evaluation of human bronchioalveolar lavage (BAL) fluid following controlled in vivo exposure to ozone to gain greater insight into its pulmonary effects. In a two-arm cross-over study, healthy adult human volunteers were randomly exposed to filtered air (FA) and to 0.3 ppm ozone for 2 hr. Bronchoscopy was performed and BAL obtained at 1 (n = 9) or 24 (n = 23) hr post-exposure. Metabolites were detected using ultrahigh performance liquid chromatography-tandem mass spectroscopy. At 1-hr post-exposure, 28 metabolites had differential concentrations (p < 0.05) in ozone-exposed compared to FA-exposed. Of these, 27 were higher and 1 was lower in the ozone samples in comparison to the FA samples. These significant metabolite changes were associated with increased glycolysis and antioxidant responses, suggesting acute-phase increased energy utilization as part of the cellular response to oxidative stress. At 24-hr post-exposure, 41 metabolites had differential concentrations (p<0.05). Many of the 24 hr post-exposure differential changes in metabolite concentrations occurred amongst amino acid-related metabolites which suggested altered energy metabolism. Changes associated with increased lipid membrane turnover were also observed. These later-stage changes were consistent with ongoing repair of airway tissues. The changes at 1 hr reflect responses to oxidative stress while the changes at 24 hr indicate a broader set of responses consistent with tissue repair. These results illustrate the ability of metabolomic analysis to identify mechanistic features of ozone toxicity and aspects of the subsequent tissue response.

ORD-022748	Snow, S., W. Cheng, A. Henriquez, M. Hodge, V. Bass, G. Nelson, G. Carswell, J. Richards, M. Schladweiler, A. Ledbetter, B. Chorley, K. Gowdy, H. Tong, and U. Kodavanti. Ozone-Induced Vascular Contractility and Pulmonary Injury are Differentially Impacted by Diets Enriched with Coconut Oil, Fish Oil, and Olive Oil. TOXICOLOGICAL SCIENCES. [HYPERLINK "https://doi.org/10.1093/toxsci/kfy003"]	Samantha Snow, ord, nheerl, ephd	Peer Reviewed	ACE PEP-2.

Cleared in STICS 8/18/2017. Now available online.

Impact / Purpose Statement

Fish oil, olive oil, and coconut oil dietary supplementation have several cardioprotective benefits, but it is not established if they can protect against air pollution-induced adverse effects. These data indicate that while fish oil offered protection from ozone-induced aortic vasoconstriction, it increased pulmonary injury/inflammation and impaired lipid transport mechanisms resulting in foamy macrophage accumulation, demonstrating the need to be cognizant of potential off-target pulmonary effects that might offset the overall benefit of this vasoprotective dietary supplement.

Product Description / Abstract

Fish, olive, and coconut oil dietary supplementation have several cardioprotective benefits, but it is not established if they protect against air pollution-induced adverse effects. We hypothesized that these dietary supplements would attenuate ozone-induced systemic and pulmonary effects. Male Wistar Kyoto rats were fed either a normal diet, or a diet supplemented with fish, olive, or coconut oil for 8 weeks. Animals were then exposed to air or ozone (0.8 ppm), 4h/day for 2 days. Ozone exposure increased phenylephrine-induced aortic vasocontraction, which was completely abolished in rats fed the fish oil diet. Despite this cardio-protective effect, the fish oil diet increased baseline levels of bronchoalveolar lavage fluid (BALF) markers of lung injury and inflammation. Ozone-induced pulmonary injury/inflammation were comparable in rats on normal, coconut oil, and olive oil diets with altered expression of markers in animals fed the fish oil diet. Fish oil, regardless of exposure, led to enlarged, foamy macrophages in the BALF that coincided with decreased pulmonary mRNA expression of cholesterol transporters, cholesterol receptors, and nuclear receptors. Serum microRNA profile was assessed and demonstrated marked depletion of a variety of microRNAs in animals fed the fish oil diet, several of which were of splenic origin. No ozone-specific changes were noted. Collectively, these data indicate that while fish oil offered vascular protection from ozone exposure, it increased pulmonary injury/inflammation and impaired lipid transport mechanisms resulting in foamy macrophage accumulation, demonstrating the need to be cognizant of potential off-target pulmonary effects that might offset the overall benefit of this vasoprotective supplement.

ORD-024286	Spade, D., C. Yue Bai , C. Lambright, J. Conley, K. Boekelheide , and E. Gray. Validation of an automated counting procedure for phthalate-induced testicular multinucleated germ cells. TOXICOLOGY LETTERS. Elsevier Science Ltd, New York, NY. [HYPERLINK "https://doi.org/10.1016/j.toxlet.2018.03.018" \t "doilink"]	Earl Gray, ord, nheerl, tad	Peer Reviewed	CSS 2.1.1

Cleared in STICS 12/13/2017. Now available online.

Impact / Purpose Statement

This study describes a new validated, automated method for accurate counting of abnormal germ cells in the fetal rat testis. Prior to the development of this method the data counting technique was very tedious, low-throughput and unbiased.

Product Description / Abstract

In utero exposure to certain phthalate esters results in testicular toxicity, characterized at the tissue level by induction of multinucleated germ cells (MNGs) in rat, mouse, and human fetal testis. Phthalate exposures also result in a decrease in testicular testosterone in rats. The anti-androgenic effects of phthalates have been more thoroughly quantified than testicular pathology due to the significant time requirement associated with manual counting of MNGs on histological sections. An automated counting method was developed in ImageJ to quantify MNGs in digital images of hematoxylin-stained rat fetal testis tissue sections. Timed pregnant Sprague Dawley rats were exposed by daily oral gavage from gestation day 17 to 21 with one of eight phthalate test compounds or corn oil vehicle. Both the manual counting method and the automated image analysis method identified di-n-butyl phthalate, benzyl butyl phthalate, dipentyl phthalate, and di-(2-ethylhexyl) phthalate as positive for induction of MNGs. Dimethyl phthalate, diethyl phthalate, the brominated phthalate di-(2-ethylhexyl) tetrabromophthalate, and dioctyl terephthalate were negative. The correlation between automated and manual scoring metrics was high (r = 0.923). Results of MNG analysis were consistent with these compounds' anti-androgenic activities, which were confirmed in an ex vivo testosterone production assay. We have developed a reliable image analysis method that can be used to facilitate dose-response studies for the reproducible induction of MNGs by in utero phthalate exposure.

ORD-023119	Custer, C., T. Custer, M. Etterson, P. Dummer, D. Goldberg, and J.C. Franson. Reproductive success and contaminant associations in tree swallows (Tachycineta bicolor) used to assess a Beneficial Use Impairment in U.S. and Binational Great Lakes' Areas of Concern. Ecotoxicology. Springer, New York, NY. [HYPERLINK "https://doi.org/10.1007/s10646-018-1913-9"]	Matt Etterson, ord, nheerl, med	Peer Reviewed	CSS 18.04.01

Cleared in STICS 8/31/2017. Now available online.

Impact / Purpose Statement

There has been concern over contamination in the Great Lakes and its effects on biota, especially reproductive effects, for well over a half century. The contaminants of concern in this highly industrialized urban landscape include many legacy organic chemicals such as PCBs, dioxins and furans, pesticides, trace elements, polycyclic aromatic hydrocarbons (PAHs), as well as, some newly emerging contaminants such as polybrominated diphenyl ethers and perfluorinated chemicals. Many of these chemicals have been shown to cause reduced hatching success in multiple species of birds nesting in the Great Lakes and elsewhere, because of embryo toxicity and through behavioral modification mechanisms. The Great Lakes Restoration Initiative was funded to assess current conditions and remediate, as needed, contaminated harbors and rivers across the Great Lakes at specific locations which were designated as Areas of Concern (AOCs) by the Great Lakes Water Quality Agreement (2012), first signed in 1972. The metric to quantify success is when an AOC can be delisted because all of its Beneficial Use Impairments (BUIs) have been removed. The Bird or Animal Deformities or Reproductive Problems is one of 14 BUIs and is listed as impaired at 15 AOCs. This manuscript examines this BUI with respect to Tree Swallow (Tachycineta bicolor) nesting success at AOCs across the Great Lakes. The results will assist the US and Canada to decide whether this BUI impairment has been removed.

Product Description / Abstract

During 2010-2014, tree swallow (Tachycineta bicolor) reproductive success was monitored at 68 sites across all 5 Great Lakes, including 58 sites located within Great Lakes Areas of concern (AOCs) and 10 non-AOCs. Sample eggs were collected from tree swallow clutches and analyzed for contaminants including polychlorinated biphenyls (PCBs), dioxin and furans, polybrominated diphenyl ethers, and 34 other organic compounds. Contaminant data were available for 360 of the 1249 clutches monitored. Markov chain multistate modeling was used to assess the importance of 5 ecological and 11 of the dominant contaminants in explaining the pattern of egg and nestling failure rates. Four of 5 ecological variables (female Age, Date within season, Year, and Site) were important explanatory variables. Of the 11 contaminants, only total dioxin and furan toxic equivalents (TEQs) explained a significant amount of the egg failure probabilities. Neither total PCBs nor PCB TEQs explained the variation in egg failure rates. In a separate analysis, polycyclic aromatic hydrocarbon exposure in nestling diet was significantly correlated with the daily probability of egg failure. The eight sites within AOCs which had poorer reproduction when compared to 9 non-AOC sites, the measure of impaired reproduction as define by the Great Lakes Restoration Initiative, were associated with exposure to dioxins and furans, PAHs, or depredation. Only 2 sites had poorer reproduction than the poorest performing non-AOC. Using a classic (non-modeling) approach to estimating reproductive success, 82% of nests hatched at least 1 egg, and 75% of eggs hatched.

ORD-024545	Villeneuve, D., B. Landesmann, P. Allavena, N. Ashley, A. Bal-Price, E. Corsini, S. Halappanavar, T. Hussell, D. Laskin, T. Lawrence, D. Nikolic-Paterson, M. Pallardy, A. Paini, R. Pieters, R. Roth, and F. Tschudi-Monnet. Representing the Process of Inflammation as Key Events in Adverse Outcome Pathways. TOXICOLOGICAL SCIENCES. Society of Toxicology. [HYPERLINK "https://doi.org/10.1093/toxsci/kfy047"]	Dan Villeneuve, ord, nheerl, med	Peer Reviewed	CSS 17.01.01

Cleared in STICS 12/18/2017. Now available online.

Impact / Purpose Statement

The adverse outcome pathway (AOP) framework is being used to organize knowledge about how biological perturbations at the molecular, biochemical, and cellular level can progress and translate into adverse outcomes of regulatory concern. Inflammation is a common response to both pathogens and contaminants and is associated with many types of target organ toxicity and disease. As such, inflammation is an important biological process to represent using the AOP framework. However, given the complexity of the underlying biology and the balance between damage and repair that underlies inflammatory processes, there has been a lack of consensus as to how this process should be described in the framework. The present forum article reports the results of a workshop is aimed at addressing critical questions about how best to capture and document this process, and others like it, using the AOP framework. This work is expected to improve application of this conceptual framework to a broader range of environmentally-relevant toxicities, further improving our ability to interpret and make decisions based on mechanistic data that can be obtained more efficiently and cost effectively than from traditional animal testing. This work contributes to the goals and objectives of CSS project 17.01 AOP discovery and development.

Product Description / Abstract

The US EPA is developing more cost effective and efficient ways to evaluate chemical safety using high throughput and computationally based testing strategies. An important component of this approach is the ability to translate chemical effects on fundamental biological processes like enzyme activities, gene expression, and basic cellular functions into what those effects mean to human health or ecosystem sustainability. The adverse outcome pathway framework was developed to facilitate that translation. Inflammation is a common response to both pathogens and contaminants and is associated with many types of target organ toxicity and disease. The current work focuses on how to appropriately represent inflammation-related toxicity, and the complex biology involved, using the AOP framework. This work helps to expand the scope toxicity mechanisms for which the framework can be productively applied. Inflammation is an important biological process involved in many target organ toxicities. However, there has been little consensus on how to represent inflammatory processes using the adverse outcome pathway (AOP) framework; specifically, in a consistent manner that allows inflammation to be recognized as a highly connected, central node within the global AOP network. The consideration of salient features common to the inflammatory process across tissues was used as a basis to propose three hub key events for use in AOP network development. Each event, “tissue resident cell activation”, “increased pro-inflammatory mediators”, and “leukocyte recruitment/activation” is viewed as a

hallmark of inflammation, independent of tissue, and can be independently measured. Using these proposed hub key events it was possible to link together a series of AOPs, that previously had no shared key events. Significant challenges remain with regard to accurate prediction of inflammation-related toxicological outcomes even if a broader and more connected network of inflammation-centered AOPs is developed. Nonetheless the current proposal addresses one of the major hurdles associated with representation of inflammation in AOPs and may aid fit-for-purpose evaluations of other AOPs operating in a network context.

ORD-024133	Hladik, M., S. Corsi, D. Kolpin, A. Baldwin, B. Blackwell, and J. Cavallin. Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA. ENVIRONMENTAL POLLUTION. Elsevier Science Ltd, New York, NY. [HYPERLINK "https://doi.org/10.1016/j.envpol.2018.01.013"]	Brett Blackwell, ord, nheerl, med	Peer Reviewed	CSS 17.01.01

Cleared in STICS 11/2/2017. Now available online.

Impact / Purpose Statement

Neonicotinoid pesticides are a growing concern among environmental scientists. To better understand the transport of neonicotinoid insecticides to a sensitive freshwater ecosystem, monthly samples (October 2015-September 2016) were collected from 10 major tributaries to the Great Lakes, USA. For the monthly tributary samples, neonicotinoids were detected in every month sampled and a total of five neonicotinoids were detected. At least one neonicotinoid was detected in 74% of the monthly samples with up to three neonicotinoids detected in an individual sample (10% of all samples). The most frequently detected neonicotinoid was imidacloprid (53%), followed by clothianidin (44%), thiamethoxam (22%), acetamiprid (2%), and dinotefuran (1%). Thiacloprid was not detected in any samples. The maximum concentration for an individual neonicotinoid was 230 ng/L and the maximum total neonicotinoids in an individual sample was 400 ng/L. The median detected individual neonicotinoid concentrations ranged from non-detect to 10 ng/L. Land use characteristics were significantly associated with certain neonicotinoid pesticides, indicating differential use patterns. This work helps better identify those pesticides most commonly detected and at what concentrations these occur, and will aid in assessing potential risk to ecological receptors.

Product Description / Abstract

To better understand the transport of neonicotinoid insecticides to a sensitive freshwater ecosystem, monthly samples (October 2015-September 2016) were collected from 10 major tributaries to the Great Lakes, USA. For the monthly tributary samples, neonicotinoids were detected in every month sampled and a total of five neonicotinoids were detected. At least one neonicotinoid was detected in 74% of the monthly samples with up to three neonicotinoids detected in an individual sample (10% of all samples). The most frequently detected neonicotinoid was imidacloprid (53%), followed by clothianidin (44%), thiamethoxam (22%), acetamiprid (2%), and dinotefuran (1%). Thiacloprid was not detected in any samples. The maximum concentration for an individual neonicotinoid was 230 ng/L and the maximum total neonicotinoids in an individual sample was 400 ng/L. The median detected individual neonicotinoid concentrations ranged from non-detect to 10 ng/L. The detections of clothianidin and thiamethoxam significantly increased as the percent of cultivated crops in the basins increased (ñ = 0.73, P = 0.01; ñ = 0.66, P = 0.04, respectively). In contrast, imidacloprid detections significantly increased as the percent of the urbanization in the basins increased (ñ = 0.66, P = 0.03). Neonicotinoid

concentrations generally increased in spring through summer coinciding with the planting of neonicotinoid-treated seeds and broadcast applications of neonicotinoids. More spatially intensive samples were collected in an agriculturally dominated area (8 sites along the Maumee River, Ohio) twice during the spring, 2016 planting season to provide further information on neonicotinoid inputs. Three neonicotinoids were ubiquitously detected (clothianidin, imidacloprid, thiamethoxam) in all water samples collected within this basin. Maximum individual neonicotinoid concentrations was 330 ng/L and maximum total neonicotinoid concentration was 670 ng/L; median detected individual neonicotinoid concentrations were 7.0 to 39 ng/L.

ORD-022899	Thom, R., J. Gaeckle, K. Buenau, A. Borde, J. Vavrinec, L. Aston, D. Woodruff, T. Khangaonkar, and J. Kaldy. Eelgrass (Zostera marina L.) Restoration in Puget Sound: Development of a Site Suitability Assessment Process. RESTORATION ECOLOGY. Blackwell Publishing, Malden, MA. [HYPERLINK "https://doi.org/10.1111/rec.12702"]	Jim Kaldy, ord, nheerl, wed	Peer Reviewed	SSWR 4.02B

Cleared in STICS 10/4/2017. Now available online.

Impact / Purpose Statement

Because of seagrass' important ecological roles, eelgrass conservation and restoration has been a major focus of the Puget Sound Partnership. As part of this project, we developed an approach to assess suitability of 2,630 sites throughout Puget Sound to support eelgrass restoration to meet the State of Washington's goal of 20% more eelgrass by 2020. This approach uses an integrated numerical biomass production model, empirical and spatial datasets, field studies, test plantings, and input from resource managers to evaluate suitability of the sites for successful eelgrass transplanting. The models identified an excess of 2,669ha with very high, high and moderate growth potential, which is above the State of Washington's 4,200ha restoration goal. This project also developed recommendations for further evaluation of sites to assess local transplant suitability and produced maps, for local and regional environmental decision makers. These recommendations, maps and models provide information on restoration potential for all sites within Puget Sound.

Product Description / Abstract

The restoration of eelgrass (Zostera marina L.), a critical ecosystem component and 'vital sign' indicator of ecosystem health, is a high priority in Puget Sound, Washington, United States. In 2011, the state set a restoration target to increase eelgrass area by 4,200 ha, a 20% increase over the present 21,500 ha by 2020. In a region as large and complex as Puget Sound, locating areas to restore eelgrass effectively and efficiently is challenging. We developed a modeling and screening process to identify and test potential restoration sites. The spatially explicit analysis of habitat suitability incorporated information from predictive models of hydrodynamics and eelgrass biomass, shoreline manager input, historical and current eelgrass area, substrate, stressors, test plantings, and monitoring of light, temperature, and survival. We evaluated 2,630 sites from which we identified 6,292 ha of highly to very highly suitable conditions for potential eelgrass habitat, which provides ample area for restoring eelgrass to meet the 20% target. Test plantings were successful at four of nine areas, which indicated significant site-specific data needs to improve predictive capability and that the capacity for Puget Sound for eelgrass may be less than that predicted by the model. We summarized the results of our analysis on maps of habitat suitability for the majority of the ~3,220 km of shoreline in Puget Sound, which serve as a starting point

for restoration site selection and planning. Our approach provided the underpinnings for a strategy for eelgrass restoration employed by Washington state agencies, which highlights information needs and potential management actions to reduce stressors and increase eelgrass cover to meet the challenging restoration goal.

ORD-023613	Soucek, D., D. Mount, A. Dickinson, and R. Hockett. Influence of dilution water ionic composition on acute major ion toxicity to the mayfly Neocloeon triangulifer. ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY. Society of Environmental Toxicology and Chemistry, Pensacola, FL. [HYPERLINK "https://doi.org/10.1002/etc.4072"]	Dave Mount, ord, nheerl, med	Peer Reviewed	SSWR 3.03A

Cleared in STICS 3/2/2018. Now available online.

Impact / Purpose Statement

This manuscript examines the acute toxicity of major ion salts to a species of mayfly, Neocloeon triangulifer. Analysis of data from natural streams with elevated major ion concentrations have indicated that mayflies are a group of organism with generally high sensitivity to major ion enrichment, but the very limited number of mayfly species that have been adapted for use in laboratory toxicity experiments creates a gap in the understanding of how sensitive this group is to specific major ion salts, and how that sensitivity is influenced by the background composition of the water in which they are exposed. Mayfly were exposed to chloride and sulfate salts of sodium, magnesium, calcium, and potassium in a range of dilution water representing different background hardness. In addition, toxicity was tested in waters whose composition was altered in specific ways to better understand what compositional factors influence response. Data from these experiments will fill an important gap in understanding how to assess and predict the toxicity of major ions to aquatic communities.

Product Description / Abstract

Both field and laboratory studies have shown that mayflies (Ephemeroptera) tend to be relatively sensitive to elevated major ion concentrations, but very little is known about how ionic composition influences these responses. The present study evaluated the toxicity of various major ion salts to the mayfly Neocloeon triangulifer over a range of background water quality conditions. The mayfly was particularly sensitive to Na2SO4, with the median lethal concentration (LC50) of 1,338 mg SO4/L being lower than LC50s reported for seven other species at that hardness. Increasing hardness of the dilution water from 30 to 150 mg/L (as CaCO3; all ions increasing proportionally) resulted in doubling of LC50s for sodium salts, and a ~1.5 fold increase in LC50 for MgSO4. Potassium salt toxicity was not strongly influenced by hardness, consistent with findings for other species. When hardness was held constant, but Ca:Mg ratio was manipulated, the ameliorative effect (relative to Ca activity) on Na2SO4 and NaCl did not appear as strong as when hardness (and other ions) were varied, but for MgSO4 the amelioration relative to Ca activity was similar between the two experiments. The toxicity of K salts to N. triangulifer was very similar to Na salts on a mM basis, which contrasts with several other species for which K salts have been found much more toxic. In addition, the toxicity of KCl to N. triangulifer was not notably affected by the co-occurring Na concentration, as has been shown for Ceriodaphnia dubia. Finally, plotting LC50s in terms of individual ion activity (Cl, SO4, Na, Mg, or K) over the range of Ca activities in dilution water resulted in significant positive relationships, with comparable slopes to those previously observed for C. dubia over the same range of Ca activities.

NHEERL Publications Accepted by Journal
March 19-25, 2018

ORD-023890	Ankley, G., and S. Edwards. The adverse outcome pathway: A multifaceted framework supporting 21st century toxicology. Current Opinion in Toxicology. Elsevier BV, AMSTERDAM, NETHERLANDS,	Gerald Ankley, ord, nheerl, med	Peer Reviewed	CSS 17.01.01

Cleared in STICS 10/19/2017. Accepted by journal 3/14/2018.

Impact / Purpose Statement
The adverse outcome pathway (AOP) framework is designed to support the use of alternative data streams, such as molecular and biochemical measurements, in chemical risk assessments. There are several examples as to how AOPs have been used in this manner, but many of these applications have not previously been summarized. This short review describes the conceptual basis of the AOP framework and presents four different case examples of its application in both human health and ecological assessments.

Product Description / Abstract
The adverse outcome pathway (AOP) framework serves as a knowledge assembly, interpretation, and communication tool designed to support the translation of pathway-specific mechanistic data into responses relevant to assessing and managing risks of chemicals to human health and the environment. As such, AOPs facilitate the use of data streams often not employed by risk assessors in the past, including information from in silico models, in vitro assays and short-term in vivo tests with molecular/biochemical endpoints. This translational capability can increase the capacity and efficiency of safety assessments both for single chemicals and chemical mixtures. Our mini-review describes the conceptual basis of the AOP framework and aspects of its current status relative to use by toxicologists and risk assessors; as part of this, four illustrative examples of different applications are provided. Finally, we provide a brief synopsis of future areas of emphasis relative to the framework.

ORD-022970	Villeneuve, D., M. Angrish, M. Fortin, I. Katsiadaki, M. Leonard, L. Margiotta-Casaluci, S. Munn, J. O'Brien, N. Pollesch, C. Smith, X. Zhang, and D. Knapen. Adverse outcome pathway networks II: Network analytics. ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY. Society of Environmental Toxicology and Chemistry, Pensacola, FL.	Dan Villeneuve, ord, nheerl, med	Peer Reviewed	CSS 17.01.01

Cleared in STICS 10/12/2017. Accepted by journal 2/26/2018.

Impact / Purpose Statement
The US EPA is developing more cost effective and efficient ways to evaluate chemical safety using high throughput and computationally based testing strategies. An important component of this approach is the ability to translate chemical effects on fundamental biological processes like enzyme activities, gene expression, and basic cellular functions into what those effects mean to human health or ecosystem

sustainability. The adverse outcome pathway framework was developed to facilitate that translation. The current presentation focuses on how to apply that framework to predict more complex interactions resulting from exposure to chemicals that cause multiple biological effects in an organism or exposures to mixtures of chemicals. It summarizes results of an expert workshop and lays out fundamental concepts that are expected to guide the analysis of AOP networks to support their application in research, risk assessment, and regulatory decision-making. This is foundational research aimed at addressing the challenges to predictive risk assessment that are posed by exposure to multiple chemicals, pleiotropic effects of single chemical exposures, and the diversity of effects chemicals may cause in different taxa, life-stages, or sexes of organisms. This research directly supports Task 2.3 under CSS Project 17.01.

Product Description / Abstract

The US EPA is developing more cost effective and efficient ways to evaluate chemical safety using high throughput and computationally based testing strategies. An important component of this approach is the ability to translate chemical effects on fundamental biological processes like enzyme activities, gene expression, and basic cellular functions into what those effects mean to human health or ecosystem sustainability. The adverse outcome pathway framework was developed to facilitate that translation. The current work focuses on how to apply that framework to predict more complex interactions resulting from exposure to chemicals that cause multiple biological effects in an organism or exposures to mixtures of chemicals. This is critical work, as most real-world exposures to chemicals involve these more complex scenarios. Exposures to multiple stressors and knowledge that stressors may have more than one effect on biological systems and those effects may vary depending on the life-stage, sex, and taxa exposed and/or the route by which those exposures occur, represent a significant challenge in risk assessment. The adverse outcome pathway (AOP) framework was designed to allow for development of AOP networks that could aid the analysis of pleiotropic and interactive effects for these complex exposure scenarios. The present paper introduces nascent concepts related to analysis of AOP networks. Graph theory-based approaches for identifying important topological features are illustrated using two example AOP networks derived from extant AOP descriptions. Considerations for identifying the most significant paths through an AOP network from either a biological or risk assessment perspective are described. Finally, approaches for identifying interactions among AOPs that may result in additive, synergistic, or antagonistic responses, or previously undefined emergent patterns of response, are introduced. Along with a companion article (Knapen et al. part I), these concepts set the stage for development of tools and case studies that will facilitate more rigorous analysis of AOP networks and evaluation of the utility of AOP network-based predictions for use in research and regulatory decision-making. This addresses one of the major themes identified through a SETAC Horizon Scanning effort focused on advancing the AOP framework.

ORD-022298	O'Malia, E., L. Johnson, and J. Hoffman. Pathways and places associated with nonindigenous aquatic species introductions in the Laurentian Great Lakes. HYDROBIOLOGIA. Springer, New York, NY, USA.	Joel Hoffman, ord, nheerl, med	Peer Reviewed	SSWR 3.01A

Cleared in STICS 7/20/2017. Accepted by journal 2/16/2018.

Impact / Purpose Statement

To allow for a timely and cost-effective management response, early detection of aquatic invasive species should occur when the species is relatively rare and not yet widespread. However, surveillance for rare species is costly and time-intensive. Knowing where invasive species are likely to be introduced can improve surveillance efficiency. We examined how proxies for three major introduction pathways (commercial shipping, recreational boating, animals in trade) are associated with detections of select nonindigenous aquatic species (NAS) across the Great Lakes. We found that the best predictor of detecting NAS is city size, highlighting the important role of cities in terms of introduction and surveillance. We also found subtle shifts in the data suggesting aquarium species are a growing concern relative to NAS introduced by the other two pathways.

Product Description / Abstract

To evaluate what types of places historically have been susceptible to NAS introduction and establishment within the Great Lakes, we developed and evaluated geospatial metrics of three prominent anthropogenic introduction pathways: commercial maritime traffic, recreational maritime traffic, and live release from urban areas. Logistic and linear regression analyses were conducted between species presence/detections and introduction pathway intensity (i.e., number of vessel trips received by a port) for 23 NAS over a five-decade period (1970 2013) to explain the apparent spatial and temporal patterns of historical aquatic invasions. The probability of NAS detections increased with increasing city size, commercial maritime trips, and marina size for all NAS, decades, and pathway combinations. City population size was the best model factor and potential proxy of NAS presence, even for NAS introduced through ballast water discharge. Through time, city population size was an increasingly significant predictor of the presence of organisms in trade, signaling a change in both the types of organisms introduced and kinds of places where introductions are occurring. Nonetheless, city population size, total commercial maritime trips, and marina size may be reasonable proxies for propagule pressure given the significant relationships between these specific pathway metrics and NAS detections.

NHEERL Publications Cleared in STICS
March 19-25, 2018

ORD-024037 Adv Notification	Flynn, K., D. Lothenbach, F. Whiteman, D. Hammermeister, M. Etterson, J. Swintek, and R. Johnson. The effects of continuous diazinon exposure on growth and reproduction in Japanese medaka using a modified Medaka Extended One Generation Reproduction Test (MEOGRT). AQUATIC TOXICOLOGY. Elsevier Science Ltd, New York, NY, USA,	Kevin Flynn, ord, nheerl, med, stb	Peer Reviewed	CSS18.04.01

Impact / Purpose Statement
The manuscript presents the use of a modified version of the EPA's Medaka Extended One Generation Reproduction Test (MEOGRT) to describe the effects of diazinon on long-term survival, growth, and reproduction in this fish species. Diazinon exposure in the low to mid- ug/L concentration range and lasting for the entire lifecycle of the fish reduced growth and reproductive output. In addition, the manuscript describes the advantages of additional growth measurements to better define the growth trajectories of exposed fish.

Product Description / Abstract
The Medaka Extended One Generation Reproduction Test (MEOGRT) is part of the U.S. Environmental Protection Agency's (USEPA) Endocrine Disruptor Screening Program (EDSP) designed to characterize the potential adverse effects to fish of a chemical through endocrine disruption. As such, the MEOGRT focuses primarily on adverse effects to reproduction while collecting some information regarding effects on growth, survival, and endocrine-related endpoints. However, the risk assessment process for fish, as mandated by legislation such as Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) or the Toxic Substances Control Act (TSCA), could benefit from a more detailed assessment of effects on growth. Typically, fish growth data in support of risk assessment is obtained from full life-cycle tests or early life stage tests using the fathead minnow. As an alternative to these tests, a modified MEOGRT was conducted to assess the effects of diazinon on the various parameters measured in the MEOGRT. Diazinon is an organophosphate insecticide that is detected in the environment and whose efficacy is a result of inhibition of the acetylcholine esterase enzyme at neuromuscular junctions and synapses of the nervous system. Diazinon at 2.9, 5.2, 10.3, 19.8, and 40.2 ìg/L was tested with the MEOGRT protocol, and the lowest observable effect concentrations of 2.9 ìg/L for fecundity and 5.2 ìg/L for growth were determined. Also, additional growth measurements were added to the MEOGRT protocol to more robustly define growth and its impacts on reproductive performance. Size starting at the first measurement day, 21 days post-fertilization, and continuing through the duration of the test was reduced with exposure to 5.2 ìg/L and above, and asymptotic size predicted from the growth modeling was reduced at 10.3 ìg/L and above.

ORD-024415	Nacci, D., A. Kuhn, J. Grear, G. Thursby, and J. Copeland. Predicting population risks from multiple stressors: mercury contamination and habitat alteration on nesting common loons. Advances in Environmental Research.	Diane Nacci, ord, nheerl, aed, peb	Book Chapter	CSS18.04.01

Impact / Purpose Statement

The approach and methods described in this book chapter illustrate a scientific process for ecological risk assessment applicable to any category of wildlife or combination of stressors. The data rich example that was selected addresses an important environmental problem, and provided an opportunity to test results for consistency with wild populations influenced by multiple, concurrent stressors. This form of model testing produces confidence in the usefulness of these methods generically to develop risk-based determinations that support the development of regulatory criteria at the state, regional or national levels. General impacts from this contribution include improved understanding by managers and scientists of links between human activities, natural dynamics, ecological stressors and ecosystem condition.

Product Description / Abstract

This chapter describes the first demonstration of a logical framework developed by the US Environmental Protection Agency to integrate best available data, methods and models to estimate risks to wildlife populations from multiple stressors. This approach responds to the need for greater realism and ecological significance in the estimation of the effects on wildlife of human-mediated stressors. Specifically, this approach provides a mechanism to link quantitatively adverse outcomes at the population level and increasing levels of specific single or a combination of multiple stressors. This demonstration was applied to estimate risks to the common loon (Gavia immer) from dietary mercury and nesting habitat degradation, over a range of realistic stressor levels. Information from field observations, controlled studies, and environmental data were used to estimate demographic parameters, and to develop biological response models for mercury and habitat suitability for loons nesting in the state of New Hampshire, USA, where the common loon is listed as a “threatened species”. Stressor-specific biological response models were used to modify population model parameters (developed from a reference population), and models were used to project quantitative population-level responses for varying combinations of environmentally-realistic stressor levels. Site-specific model projections and field observations were generally coherent, suggesting the usefulness of this approach as a basis for management decisions to mitigate the effects of chemicals and other human-mediated stressors.

ORD-025898	Magee, T., K. Blocksom, A. Herlihy, and A. Nahlik. Characterizing the extent of an indicator of potential stress from nonnative plants in wetlands across the conterminous US. ENVIRONMENTAL MONITORING AND ASSESSMENT. Springer, New York, NY, USA,	Mary Kentula, ord, nheerl, wed, feb	Peer Reviewed	SSWR3.01A

Impact / Purpose Statement

A critical need for continental-scale wetland assessment is an effective biological indicator of ecological stress, comprised of easy-to-measure metrics, that is relevant across large scales and wetland types. To address this need, we developed the Nonnative Plant Indicator (NNPI), a categorical indicator of potential stress (low, moderate, high, or very high) from the set of nonnative plants occurring at a given location. NNPI potential stress categories are based on a decision matrix using exceedance values for three metrics: richness, relative frequency, and relative cover of nonnative plants. We applied the NNPI to wetlands across the conterminous United States using data from the USEPA 2011 National Wetland Condition Assessment (NWCA). NNPI status from 967 NWCA probability sites was used to estimate wetland area within the four NNPI categories at national, ecoregional, and wetland type scales. Estimates of wetland area with low, moderate, high, and very high NNPI will be useful to policy makers and resource managers in identifying where potential stress from nonnative plants may be most extensive, prioritizing management actions, and informing future research. To help understand NNPI extent patterns, we conducted and reported on exploratory analyses characterizing: 1) the complement of individual nonnative species observed at sampled sites, 2) ecoregional and wetland type patterns for NNPI metrics and nonnative growth habit groups, 3) ecoregional and wetland type characteristics (native vegetation, abiotic properties, and human-mediated disturbance) and 4) potential ecological predictors of NNPI status. This research also contributes to work under SSWR Task 3.01A, subtask 1.1.

Product Description / Abstract

Nonnative plants are recognized as stressors to wetlands and other ecosystems, competing with native plant species or altering ecosystem processes and may affect ecological condition, posing challenges to resource managers. We devised the Nonnative Plant Indicator (NNPI) as a categorical indicator of potential stress to ecosystems from the collective set of nonnative plant taxa occurring at a particular location. The NNPI indicates categories of potential stress from nonnative plants (low, moderate, high, or very high), based on a decision matrix using exceedance values for three metrics: richness, relative frequency, and relative cover of nonnative plants. We applied the NNPI to wetlands across the conterminous United States (US) using plant data collected during the US Environmental Protection Agency’s 2011 National Wetland Condition Assessment (NWCA). Based on the NWCA probability design, wetland area of the sampled population occurring in each NNPI category was estimated at the national-scale, and within five large ecoregions and four broad wetland types. Estimates for wetland area in different NNPI categories are expected to be useful to policy makers or resource managers in identifying situations where potential stress from nonnative plants may be most extensive, which may help inform and prioritize management actions and future research. At the national-scale, potential stress from nonnative plants as indicated by NNPI category was low for approximately 61% (~15.3 million ha), moderate for about 20% (~5.2 million ha), high for about 10% (~2.48 million ha), and very high for about 9% (~2.2 million ha) of the sampled population wetland area. The percent of wetland area in the sampled population with high and very high NNPI was greater within interior and western ecoregions (~29 to 87%) than within ecoregions in the eastern half of the nation (~11%). Among wetland types, greater percent area with high and very high NNPI was also observed for herbaceous vs. woody wetland types, and for inland vs. estuarine wetland types. To aid in understanding the patterns

described by the NNPI extent results, we also conducted, and report on results for, a series of exploratory analyses to: 1) characterize the complement of individual nonnative species (n = 443) observed across 1138 sampled sites, 2) examine ecoregional and wetland type patterns for the three NNPI metrics and for growth habit groups of nonnative plants, 3) describe ecoregional and wetland type characteristics based on (native vegetation attributes, abiotic properties, and human-mediated disturbance) to broadly characterized the areas into which nonnative plants are invading, and 4) identify potential predictors of NNPI status.

ORD-025629	de Vries, P., M. Sabater-Lleal, J. Huffman, J. Marten, N. Pankratz, T. Bartz, H. de Haan, G. Delgado, J. Eicher, A. Martinez, C. Ward-Caviness, J. Brody, M. Chen, M. de Maat, M. Franberg, M. Kleber, K. Liu, F. Rivadeneira, J. Soria, W. Tang, G. Tofler, A. Uitterlinden, A. Vlieg, E. Boerwinkle, N. Davies, O. Franco, B. McKnight, B. Psaty, A. Reiner, M. Fornage, A. Hamsten, W. Maerz, F. Rosendaal, J. Souto, A. Dehghan, A. Johnson, A. Morrison, C. O'Donnell, and N. Smith. A genome-wide association study identifies new loci for Factor VII and implicates Factor VII in the etiology of ischemic stroke.. BLOOD. American Society of Hematology, Washington, DC, USA,	Cavin Ward-Caviness, ord, nheerl, ephd, crb	Peer Reviewed	N/A

Impact / Purpose Statement

This manuscript describes a large, international effort to uncover the genetic basis of Factor VII and to understand the role that Factor VII plays in ischemic stroke. Studies such as these help to uncover genes and genetic risk variants for disease and molecular factors, many of which are associated with environmental factors.

Product Description / Abstract

Factor VII (FVII) is an important component of the coagulation cascade. Few genetic loci regulating FVII activity and/or levels have been discovered to date. We conducted a meta-analysis of nine genome-wide association studies of plasma FVII levels (seven FVII activity and two FVII antigen) among 27,495 participants of European and African ancestry. Each study performed ancestry-specific association analyses. Inverse variance weighted meta-analysis was performed within each ancestry group and then combined for a trans-ancestry meta-analysis. Our primary analysis included the seven studies that measured FVII activity, and a secondary analysis included all nine studies. We provided functional genomic validation for newly identified significant loci by silencing candidate genes in a human liver cell line (HuH7) using siRNA and then measuring F7 mRNA and FVII protein expression. Lastly, we used meta-analysis results to perform Mendelian randomization analysis to estimate the causal effect of FVII activity on coronary artery disease, ischemic stroke, and venous thromboembolism. We identified two novel (REEP3 and JAZF1-AS1) and six known loci associated with FVII activity, explaining 19.0% of the variance. Adding FVII antigen data to the meta-analysis did not result in the discovery of further loci. Silencing REEP3 in HuH7 cells upregulated FVII, while silencing JAZF1 downregulated FVII. Mendelian randomization analyses suggest that FVII activity has a positive causal effect on the risk of ischemic stroke. Variants at REEP3 and JAZF1 contribute to FVII activity by regulating F7 expression levels. FVII activity appears to contribute to the etiology of ischemic stroke in the general population.

ORD-025895	Wang, H., M. Taffi, C. Chion, B. Rashleigh, T. Klanjšček, L. Harris, P. Goethals, and B. Fath. Guest Editorial for Special Issue: Ecological Modelling Global Conference 2016: 20th Biennial ISEM Conference, 8 - 12 May 2016, Towson, Maryland, USA.. ECOLOGICAL MODELLING. Elsevier Science BV, Amsterdam, NETHERLANDS,	Brenda Rashleigh, ord, nheerl, rpcs	Non-Peer Reviewed	N/A

Impact / Purpose Statement

Ecological systems are comprised of complex interactions of organisms with each other and with their environments, virtually all of which are impacted by human activities. Human well-being is dependent upon, and inextricably integrated with, the functioning of ecosystems and the services they provide. Understanding the connection of the patterns we observe in the world around us to the processes generating those patterns is a prerequisite for the growth of ecology as a science and for the proactive management of ecological systems. Ecological models integrating economic and social components emphasize the coupling of human and natural systems and hold the promise of more enlightened management of both human and natural resources. Recent developments at the interfaces among ecology, mathematics, statistics, and computer science have facilitated dramatic advances in our ability to model the complexity and uncertainty of ecological systems across space and time. We hope the papers selected for inclusion in this Special Issue are indicative of the breadth of current topics being addressed via ecological modelling.

Product Description / Abstract

This Special Issue contains a collection of papers presented at The Ecological Modelling Global Conference 2016: 20th Biennial International Society of Ecological Modelling (ISEM) Conference which was held at Towson University, Maryland, United States. Over the past 40+ years, Ecological Modelling has published many special issues, some devoted to specific topics of current interest and some covering important conferences. The journal's editorial policy has encouraged open discussions on all topics relevant to ecological modelling, and, it is fair to say, has contributed substantially to development of the field. Today, the scientific community recognizes the importance of ecological modelling in both theoretical and applied studies, and society relies increasingly on ecological models to address a wide variety of environmental and natural resource management issues. This diversity of uses of ecological models is reflected in the diversity of topics covered in this Special Issue.